RECEIVED
CENTRAL FAX CENTER
SEP 21 2005

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appellant: Peter M. Glazer

Serial No.: 09/978,333 Art Unit: 1634

Filed: October 15, 2001 Examiner: Carla Myers

For: *TRIPLE-HELIX FORMING OLIGONUCLEOTIDES FOR TARGETED MUTAGENESIS*

Mail Stop Appeal Brief-Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPEAL BRIEF

Sir:

This is an appeal from the final rejection of claims 7-12 and 15-25 in the Office Action mailed May 10, 2005, in the above-identified patent application. A Notice of Appeal was filed on July 21, 2005. The Commissioner is hereby authorized to charge $250.00, the fee for the filing of this Appeal Brief for a small entity, to Deposit Account No. 50-3129. It is believed that no additional fee is required with this submission. However, should an additional fee be required, the Commissioner is hereby authorized to charge the fee to Deposit Account No. 50-3129.

09/23/2005 MBINAS 00000003 503129 09978333
01 FC:2402 250.00 DA

(1) REAL PARTY IN INTEREST

The real party in interest of this application is the assignee, Yale University, New Haven, CT.

(2) RELATED APPEALS AND INTERFERENCES

There are no directly related appeals or interferences known to appellant, the undersigned, or appellant's assignee which directly affects, which would be directly affected by, or which would have a bearing on the Board's decision in this appeal. However, the Board's attention is drawn to the appeal in U.S.S.N. 09/783,338 by the same inventor and addressing many similar issues.

(3) STATUS OF CLAIMS

Claims 7-12 and 15-25 are pending. Claims 1-6 and 13-14 have been cancelled. Claims 7-12 and 15-25 are on appeal. The text of each claim on appeal, as pending, is set forth in an Appendix to this Appeal Brief.

(4) STATUS OF AMENDMENTS

An amendment after final rejection was mailed on July 21, 2005. In the Advisory Action mailed August 11, 2005, the Examiner indicated that this amendment would be entered. The Claims Appendix sets forth the claims on appeal.

(5) SUMMARY OF CLAIMED SUBJECT MATTER

Independent claim 7 defines a method for targeted recombination of a nucleic acid molecule (for support see at least page 7, lines 12-13) comprising the steps of: a) providing a single-stranded oligonucleotide having a sequence that forms a triple-stranded nucleic acid molecule by hybridizing with a target sequence in a double-stranded nucleic acid molecule (for support see at least page 7, lines 7-9) with a Kd of less than or equal to 2×10^{-7} (for support see at least page 5, line 2) and b) providing a donor nucleic acid such that recombination of the donor nucleic acid into the target sequence is induced by triple helix formation between the single-stranded oligonucleotide and the double-stranded nucleic acid molecule (for support see at least page 7, lines 12-17).

Dependent claim 8 and 16 define the method of claim 7 and claim 15, respectively, wherein the single-stranded oligonucleotide is between 10 and 60 nucleotides in length (for support see at least page 14, lines 21-25). Dependent claim 9 defines the method of claim 7 wherein the single-stranded oligonucleotide is tethered to the donor nucleic acid (for support see at least page 7, lines 17-18). Dependent claim 10 defines the method of claim 7 wherein the double-stranded nucleic acid molecule encodes a protein and the targeted recombination of the donor nucleic acid with the double-stranded nucleic acid molecule alters the activity of the protein encoded by the double-stranded nucleic acid molecule (for support see at least page 5, lines 6-12). Dependent claim 11 and dependent claim 24 define the method of claim 7 and claim 15, respectively, wherein the double-stranded nucleic acid molecule is selected from the group consisting of a gene, an oncogene, a defective gene, a viral genome, and a portion of a viral

genome (for support see at least page 6, lines 8-16 and page 7, lines 25-29). Dependent claim 12 defines the method of claim 7 wherein the donor nucleic acid is at least 30 nucleotide residues in length (for support see at least page 9, lines 26-27).

Dependent claim 15 defines the method of claim 7 to produce changes in the genome of an intact human or animal (for support see at least page 7, lines 5-7 and page 11, lines 17-22) wherein the single-stranded oligonucleotide is administered into an intact human or animal (for support see at least page 11, lines 17-22) having a sequence that forms a triple-stranded nucleic acid molecule with the target sequence located in the genome of the intact human or animal (for support see at least page 5, lines 6-8), wherein the oligonucleotide binds to the target sequence with a Kd of less than or equal to 2 x 10-7 (for support see at least page 5, line 2), and mutates the target sequence (for support see at least page 7, lines 9-10). Dependent claim 17 defines the method of claim 15 wherein the oligonucleotide is dissolved in a physiologically acceptable carrier (for support see at least page 11, lines 17-18). Dependent claim 18 defines the method of claim 15 wherein the oligonucleotide is recombinagenic (for support see at least page 9, lines 22-25). Dependent claim 19 defines the method of claim 15 wherein the oligonucleotide stimulates recombination of an exogenously supplied donor nucleic acid with the target sequence of the genome (for support see at least page 14, lines 3-5). Dependent claim 20 defines the method of claim 18 wherein the oligonucleotide stimulates recombination of a donor nucleic acid that is tethered to the oligonucleotide with the target sequence of the genome (for support see at least page 9, lines 11-14). Dependent claim 22 defines the method of claim 21 wherein the gene is a defective -hemoglobin gene, cystic fibrosis gene, xeroderma pigmentosum gene, nucleotide

excision repair pathway gene, or hemophilia gene (for support see at least page 12, lines 15-18 and page 13, lines 8-11). Dependent claim 23 defines the method of claim 15 wherein the oligonucleotide is composed of homopurine or homopyrimidine nucleotides (for support see at least page 8, lines 3-4). Dependent claim 24 defines the method of claim 15 wherein the oligonucleotide is composed of polypurine or polypyrimidine nucleotides (for support see at least page 8, lines 4-5). Dependent claim 25 defines the method of claim 9 wherein the donor nucleic acid is between 10 and 40 nucleotides (for support see at least page 8, line 1).

(6) GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The issues presented on appeal are:

(1) whether claims 7-12 and 15-25 are enabled as required by 35 U.S.C. § 112, first paragraph.

(2) whether claims 19 and 20 are definite as required by 35 U.S.C. § 112, second paragraph.

(3) whether claims 7-12, 15-21 and 23-25 are novel as required by 35 U.S.C. § 102(b) over Chan, et al., *Journal of Biological Chemistry* 274:11541-11548 (1999) ("Chan").

(4) whether claim 22 is non-obvious as required by 35 U.S.C. § 103(a) over Chan.

(7) GROUPING OF CLAIMS

The claims do not stand or fall together as discussed below.

(8) ARGUMENT

(i) Rejections under 35 U.S.C. § 112, first paragraph

The Legal Standard for Enablement

The Court of Appeals for the Federal Circuit (CAFC) has described the legal standard for enablement under § 112, first paragraph, as whether one skilled in the art could make and use the claimed invention from the disclosures in the patent coupled with information known in the art without undue experimentation. See, e.g., *Amgen v. Hoechst Marion Roussell* 314 F.3d 1313 (Fed. Cir. 2003) and*Genentech, Inc. v. Novo Nordisk A/S*, 108 F3d at 165, 42 USPQ2d at 1004 (quoting *In re Wright*, 999 F.2d 1557, 1561, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). See also *In re Fisher*, 427 F.2d at 839, 166 USPQ at 24; *United States v. Telectronics, Inc.*, 857 F.2d 778 (Fed. Cir. 1988); and *In re Stephens*, 529 F.2d 1343 (CCPA 1976). The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation. *M.I.T. v. A.B. Fortia*, 774 F.2d 1104 (Fed. Cir. 1985). In addition, as affirmed by the Court in *Spectra-Physics, Inc. v. Coherent, Inc.*, 827 F.2d 1524 (Fed. Cir. 1987), a patent need not teach, and preferably omits, what is well known in the art.

Whether the disclosure is enabling is a legal conclusion based upon several underlying factual inquiries. See *In re Wands*, 858 F.2d 731, 735, 736-737, 8 USPQ2d 1400, 1402, 1404 (Fed. Cir.1988). As set forth in *Wands*, the factors to be considered in determining whether a claimed invention is enabled throughout its scope without undue experimentation include the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art,

the relative skill of those in the art, the predictability or unpredictability of the art, and the breadth of the claims. In cases that involve unpredictable factors, "the scope of the enablement obviously varies inversely with the degree of unpredictability of the factors involved." *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). The fact that some experimentation is necessary does not preclude enablement; what is required is that the amount of experimentation ' must not be unduly extensive.' *In re Atlas Powder Co., v. E.I. DuPont De Nemours & Co.* , 750 F.2d 1569, 1576, 224 USPQ 409, 413 (Fed. Cir.1984).

As noted in *Ex parte Jackson*, the test is not merely quantitative, since a considerable amount of experiment is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to practice a desired embodiment of the invention claimed. See *Ex parte Jackson*, 217 USPQ 804, 807 (PTO Bd. App. 1982). There is no requirement for examples.

The Claimed Method

Appellant has discovered that administration of a triple helix forming oligonucleotide (or "TFO") in combination with a DNA fragment or "donor nucleic acid" promotes site-specific targeted recombination of the donor nucleic acid into a target region. The DNA fragment may be tethered to the TFO or not as described in the specification at least at page 7, lines 12-19. Methods for insertion of a homologous DNA sequence or DNA fragment *in vivo* were known to one of ordinary skill in the art and are described in the specification at least at page 3, lines 2-19. However, these methods are limited in their efficacy. Methods of targeting TFOs to a specific

site in the genome were known to one of ordinary skill in the art and are described in the specification at least at page 2, lines 1-5. It is understood by one of ordinary skill in the art that TFOs have at most two targets in the genome since there are only 2 copies of every chromosome. Single-stranded oligonucleotides having a sequence that forms a triple-stranded molecule by hybridizing with a target sequence in a double-stranded nucleic acid molecule are described in the specification at least at page 7 to page 11. Methods for targeted recombination of a target sequence using a single-stranded oligonucleotide as described in the specification at least at pages 7-11 and a donor nucleic acid such that the donor nucleic acid is recombined into the target sequence is described in the specification at least at page 9, lines 11-28 and page 14, lines 8-25. Methods of tethering or linking a donor nucleic acid to the single stranded oligonucleotide are described in the specification at least at page 17, lines 19-26. Methods for administration of the single-stranded oligonucleotide and donor nucleic acid *in vitro* and *in vivo* are described in the specification at least at page 11, line 17 to page 12, line 13 and at page 14, lines 8-19. As described in the specification at least at page 11, lines 22-24, nucleic acid molecules are taken up by cells and tissues in animals such as mice without special delivery methods, vehicles or solutions. The *in vivo* distribution of oligonucleotides has been studied with a variety of modified oligonucleotides in mice (Zendegui, et al., *Nucleic Acids Research* 20:307-314 **(1992)** and Agrawal, et al., *Proc. Natl. Acad. Sci.* USA 88, 7595 **(1991)**, submitted with Appellant Amendment and Response filed July 21, 2005, copies of which are enclosed). **These studies established that DNA molecules can be administered by i.p. or intravenous injections and gain access to tissues (outside the central nervous system) and to cell nuclei.** As expected

from these studies, chromosomal DNA throughout the somatic tissues of an animal can be targeted by nucleic acids, which is also described in the specification at least at examples 6 and 7, pages 31-35.

Claims 7-12 are enabled

An analysis of the Wands factors clearly demonstrates that claims 7-12 are enabled by the specification of the present application.

Independent claim 7 defines a method for targeted recombination of a nucleic acid molecule. In contrast to claims 15-25, claims 7-12 do not specify where the nucleic acid molecules are. The nucleic acid molecules may be *in vitro* in a cell free solution. The nucleic acid molecules may be in isolated cells, for example, obtained by biopsy, from a bone marrow sample, or from blood. The nucleic acid molecules may be in cells in tissue or a patient. Limitations directed to the *in vivo*, *ex vivo*, and *in vitro* applications of the method as defined by claims 7-12 have been continually read into the claims by the Examiner but are not in fact present in the claim language. There is no argument that the claims are enabled for delivery *in vitro* to either a cell free system or *ex vivo* to isolated cells. Therefore claims 7-12 are enabled.

The Wands Factors

(i) Quantity of experimentation, the amount of direction or guidance presented in the specification, and presence of working example.

Since *in vivo* distribution of nucleic acids is achieved by injection of nucleic acids, all that is required to practice the method as defined by the claims is

(1) provision of a suitable TFO and donor nucleic acid and

(2) injection of the TFO and donor nucleic acid. Therefore, it is clear that the quantity of experimentation required to practice the claimed method is minimal. It is also clear from the amount of guidance provided in the specification as discussed above that the amount of experimentation required to practice the claimed method is not undue.

(ii) the state of the prior art, relative skill of those in the art, and the predictability of the art.

Given the state of the art as discussed above the relative skill of those in the art was high and there was predictability in the art. Methods for insertion of a homologous DNA sequence or DNA fragment *in vivo* and methods of targeting TFOs to a specific site in the genome were known to one of ordinary skill. The specification at least at pages 21-22 and Table 1discloses that TFOs result in site-directed mutagenesis *in vitro*. Given the knowledge of one of ordinary skill in the art that for *in vivo* distribution of nucleic acids all that is required is injection of the nucleic acids, one of ordinary skill in the art would expect that a TFO injected into an animal would result in site-directed mutagenesis as predicted by the *in vitro* data.

Indeed this is exactly what Appellant demonstrates in Examples 6 and 7: injection of the TFO resulted in site-directed mutagenesis *in vivo* as predicted from the *in vitro* data. There has been no evidence provided by the examiner that the evidence in the specification would not be predictive of an oligonucleotide which further included a donor nucleic acid. The evidence in the specification clearly demonstrates efficacy in a cell system which was predictive of the actual efficacy in animals. Appellant has provided similar results for the oligonucleotides linked or unlinked to a donor nucleic acid using both cell-free and cell systems (see Examples 2-4 in the

specification at pages 27-31 and page 6, lines 17-22 citing Luo, et al., "High-frequency intrachromosomal gene conversion induced by triplex-forming oligonucleotides microinjected into mouse cells" *PNAS* 97(16):9003-9008 (2000) a copy of which was enclosed with the Amendment and Response filed March 1, 2005). Absent some evidence otherwise, one skilled in the art would expect the oligonucleotides, linked or unlinked to a donor nucleic acid, to have the same degree of efficacy in animals as in the cell systems, based on the evidence in the specification. One of ordinary skill in the art would expect that injection of nucleic acid molecules as defined by the claims into an animal would result in distribution of the nucleic acid molecules to tissues and cell nuclei, thereby resulting in targeted recombination of a donor nucleic acid into a target DNA sequence. Therefore, it would not require undue experimentation to practice the method for targeted recombination *in vivo* as defined by claims 7-12.

Claims 15-25 are enabled

An analysis of the Wands factors clearly demonstrates that claims 15-25 are enabled by the specification of the present application. Dependent claims 15-25 specify that the method of claim 7 is carried out to produce changes in the genome of an intact human or animal.

The Wands Factors

(i) Quantity of experimentation, the amount of direction or guidance presented in the specification, and presence of working example.

As discussed above, studies have established that DNA molecules can be administered by i.p. or intravenous injections and will gain access to tissues and cell nuclei. As expected from these studies, Appellant demonstrated that chromosomal DNA throughout the somatic tissues of

an animal can be targeted by nucleic acids, which is described in the specification at least at examples 6 and 7, pages 31-35. Since *in vivo* distribution of nucleic acids is achieved by injection of nucleic acids and all that is required to practice the method as defined by the claims is injection of a TFO linked or unlinked to a donor nucleic acid, it is clear that the quantity of experimentation required to practice the claimed method is minimal. It is also clear from the amount of guidance provided in the specification as discussed above that the amount of experimentation required to practice the claimed method is not undue.

(ii) The state of the prior art, relative skill of those in the art, and the predictability of the art.

Methods for insertion of a homologous DNA sequence or DNA fragment *in vivo* and methods of targeting TFOs to a specific site in the genome were known to one of ordinary skill in the art. The specification at least at pages 21-22 and Table 1discloses that TFOs result in site-directed mutagenesis *in vitro*. Given the knowledge of one of ordinary skill in the art that for *in vivo* distribution of nucleic acids all that is required is injection of the nucleic acids, one of ordinary skill in the art would expect that a TFO injected into an animal would result in site-directed mutagenesis as predicted by the *in vitro* data. Indeed this is exactly what Appellant demonstrates in Examples 6 and 7. As discussed above, there has been no evidence provided by the examiner that the evidence in the specification would not be predictive of an oligonucleotide which further included a donor nucleic acid. Appellant has provided similar results for the oligonucleotides linked or unlinked to a donor nucleic acid using both cell-free and cell systems. Absent some evidence otherwise, one skilled in the art would expect the oligonucleotides linked

or unlinked to a donor nucleic acid to have the same degree of efficacy in animals as in the cell systems, based on the evidence in the specification. Therefore, it would not require undue experimentation to practice the method for targeted recombination *in vivo* as defined by claims 15-25.

Evidence Cited by the Examiner

All the evidence presented by Appellant clearly demonstrates that TFOs induce site-specific recombination in the intended target. The examiner has presented no evidence to the contrary, only the observation, typical of scientific publications, that there might be some adverse consequences. It is important to note that although the Examiner recognizes that delivery is an issue, Appellant has established that it is not insurmountable and provided evidence showing that it is possible to practice the claimed methods. Appellant has provided ample evidence to show that the present application was such that one skilled in the art would find the specification fully enabling. The Patent Office is required to accept the truth of the Appellant's statements unless a reason to do otherwise can be substantiated. *In re Marzocchi*, 439 F.2d at 223 (CCPA 1971). The Examiner has failed to substantiate any reason why the evidence presented by the Appellant in the specification and the publications does not sufficiently overcome the Examiner's concerns.

(ii) Rejections under 35 U.S.C. § 112, second paragraph

The test for definiteness under 35 U.S.C. § 112, second paragraph, is whether "**those skilled in the art would understand what is claimed when the claim is read in light of the specification.**" *Orthokinetics, Inc. v. Safety Travel Chairs, Inc.*, 806 F.2d 1565, 1576, 1

USPQ2d 1081, 1088 (Fed. Cir. 1986). It cannot be doubted that the claim itself must not be divorced in a vacuum from the specifications and descriptions accompanying it (See, e.g., *Schering Corp v. Gilbert*, 153 F.2d 428, 432 (2d Cir. 1946)). The M.P.E.P. explains that the examiner's focus during examination of claims for compliance with the definiteness requirement "is whether the claim meets the threshold requirements of clarity and precision, *not whether more suitable language or modes of expression are available.*" (M.P.E.P. 2173.02, emphasis added) The M.P.E.P. further explains that " [s]ome latitude in the manner of expression and the aptness of terms should be permitted even though the claim language is not as precise as the examiner might desire." (*Id.*)

In the Office Action mailed September 22, 2004, claims 19 and 20 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for the phrase "DNA fragment." In response, claims 19 and 20 were amended in the Amendment and Response sent via facsimile March 1, 2005 to recite "donor nucleic acid" as recited in the claims from which claims 19 and 20 depend. In the Final Office Action mailed May 5, 2005, the Examiner maintained the rejection of claims 19 and 20 as being indefinite for the phrase "DNA fragment." In the Amendment and Response sent via facsimile on July 21, 2005, Appellant pointed out that Claims 19 and 20 were amended in the Amendment and Response of March 1, 2005 and are not indefinite because they no longer recite "DNA fragment." In the Advisory Action mailed August 11, 2005, the Examiner maintained the rejection of claims 19 and 20 for recitation of the phrase "donor nucleic acid." This is a new rejection and the Appellant has been provided with

no opportunity to respond. However, it is believed this phrase is quite clear, and would be understood by anyone skilled in the art from the specification and examples discussed above.

Claims 19 and 20, as amended, are not indefinite. It cannot be doubted that the claim itself must not be divorced in a vacuum from the specifications and descriptions accompanying it (See, e.g., *Schering Corp v. Gilbert*, 153 F.2d 428, 432 (2d Cir. 1946)). In view of the specification, it would be obvious to one of ordinary skill in the art that the donor nucleic acid as defined in claim 7 is the same donor nucleic acid as defined in claims 19 and 20. Claim 7 does not specify whether the donor nucleic acid is tethered or not tethered to the single-stranded oligonucleotide. Claim 19 which depends indirectly from claim 7 states that the donor nucleic acid **is not** tethered to the single-stranded oligonucleotide. Claim 20, which is similar to claim 9 and depends indirectly from claim 7, states that the donor nucleic acid is tethered to the single-stranded oligonucleotide. As clearly described in the specification at least at page 9, lines 11-14, the triplex forming oligonucleotides can be tethered or not to a donor nucleic acid as desired. Therefore, claims 19 and 20 are definite.

(iii) Rejections Under 35 U.S.C. § 102

Chan

Chan is not prior art as the present application is entitled to a priority date of 1995.

The present application is a continuation-in-part of U.S.S.N. 09/411,291 filed on October 4, 1999 ("the 1999 application"), which is a divisional of U.S.S.N. 08/476,712 filed on June 7, 1995 ("the 1995 application"), page 1, paragraph 1. The 1999 application, which issued as U.S. Patent No. 6,303,376 ("the '376 patent"), and the 1995 application, which issued as U.S. Patent

No. 5,962,426 ("the '426 patent"), provide support under 35 U.S.C. 120 for the claims of the present application.

The discussion below refers to the '376 patent. The disclosure of the '376 patent and the '426 patent is the same since the 1999 application which issued as the '376 patent is a divisional of the 1995 application which issued as the '426 patent and contains no new subject matter.

It should be noted that the present application differs from the earlier filed application primarily by virtue of the **examples**. Example 1, which is found in the '376 and '426 patents as well as the present application, specifically describes targeted mutagenesis by TFOs in monkey COS cells, patient derived XPA cells, patient derived XPV cells, and normal human fibroblasts. The remaining examples, 2 through 8, found only in the present application, support the findings disclosed in example 1. Example 2 describes the ability of TFOs to promote recombination in human cell-free extracts. Examples 3, 4, and 5 describe the role of recombination and repair proteins in the pathway of TFO induced recombination. Example 6 describes targeted mutagenesis by TFOs at genomic sites in somatic cells of adult mice. Example 7 describes heritable changes produced by TFO induced recombination in adult mice. Example 8 describes that induced mutagenesis is specifically brought about through triple-helix formation ***in vivo***.

It should also be noted that these examples were submitted in the prosecution of the parent application in the form of a declaration submitted under 35 U.S.C. § 1.132 by the Appellant. It was clearly made of record in the prior prosecuted application that the examples in the declaration were supportive of the disclosure, not adding new subject matter. This application has now issued.

Claims 7-12

Independent claim 7 defines a method for targeted recombination of a nucleic acid molecule. The '376 patent discloses at least at column 3, lines 1-4 that "the binding of the oligonucleotide to the target region stimulates mutations within or adjacent to the target region using cellular DNA synthesis, **recombination**, and repair mechanisms." The '376 patent also discloses that TFOs are particularly useful as a tool to cause targeted mutagenesis. In conjunction with the disclosure of the '376 patent and the knowledge of one of ordinary skill in the art that mutagenesis can result from recombination, it is clear that the '376 patent discloses that the mutations in a region of DNA targeted by a TFO can be accomplished through recombination.

The method comprises the step of providing a single-stranded oligonucleotide having a sequence that forms a triple-stranded nucleic acid molecule by hybridizing with a target sequence in a double-stranded nucleic acid molecule. The '376 patent at least at column 3, lines 58-61 discloses that single-stranded oligonucleotides bind to or hybridize with a predetermined region of a double-stranded DNA molecule so as to form a triple-stranded structure.

The oligonucleotide has a Kd of less than or equal to 2×10^{-7}. Support can be found in the '376 patent at least at column 5, lines 3-4, and at column 9, lines 25-56, and at Table 1. Table 1 states that AG20 has a Kd of 3×10^{-7} and AG 30 has a Kd of 2×10^{-8}. These results in Table 1 demonstrate that TFOs with Kd's of 3×10^{-7} or less result in mutagenesis induced by triple helix formation. Since 2×10^{-7} is less than 3×10^{-7}, Kds of less than or equal to 2×10^{-7} are supported by the '376 patent.

The method further comprises the step of providing a donor nucleic acid such that recombination of the donor nucleic acid into the target sequence is induced by triple helix formation between the single-stranded oligonucleotide and the double-stranded nucleic acid molecule. The '376 patent discloses at least at column 3, lines 49-56, that TFOs can be used to stimulate recombination of a DNA fragment into a target region. It is clear that claim 7 is fully supported by the '376 and '426 patents.

Claim 8 is supported by the '376 and '426 patents. As pointed out by the examiner on page 17 of the office action, the '376 and '426 patents disclose TFOs of 7 to 40 nucleotides (see column 4, line 4 of the '376 patent) and TFOs of 10, 20, 30 and 57 nucleotides in length. This clearly demonstrates that TFOs of between 10 to 60 nucleotides in length as defined in claim 8 is supported by the '376 and '426 patents.

Claim 9 is supported by the '376 and '426 patents. The '376 patent discloses at least at column 3, lines 49-56, that TFOs can be used to stimulate recombination of a DNA fragment into a target region, but does not distinguish between whether the DNA fragment is linked or unlinked. However, at least at the paragraph spanning column 1 to column 2, the '376 patent discloses that TFOs are useful alone or linked to reactive moieties. It would be clear to one of skill in the art that the DNA fragment described in the '376 patent at least at column 3, lines 49-56 and at column 6, lines 40-58 could be linked to the TFO as defined by claim 9.

Support for claim 10 can be found in the '376 patent at least at column 3, lines 47-56 which discloses that a mutation can activate, inactivate or alter the activity and function of a gene

containing a target site and that this mechanism can be accomplished when a TFO stimulates homologous recombination of a donor nucleic acid into the target region.

Support for claim 11 can be found in the '376 patent at least at the paragraph spanning columns 3 and 4, which describes that the double-stranded nucleic acid can be a gene, an oncogene, or a defective gene, and at column 6, lines 19-31, which describes that the double-stranded nucleic acid can be a viral genome or a portion of a viral genome.

In summary, it is clear that claims 7-12 of the present application are **fully** supported by the '376 and '426 patents. Therefore, Chan is not prior art and claims 7-12 are not anticipated by Chan.

Claims 15-25

Claim 15 defines the method of claim 7 to produce changes in the genome of an intact human or animal. The '376 patent describes methods of producing changes in the genome of a human or animal in the specification at least at column 2, lines 11-59. As mentioned above, the '376 patent discloses at least at column 3, lines 49-56, and again at column 6, lines 40-58, methods in which the TFOs can be used to stimulate homologous recombination of a DNA fragment into a target region. The method comprises the steps of administering the single-stranded oligonucleotide into an intact human or animal. The '376 patent discloses at least at column 5, lines 49-58, that the oligonucleotides are preferably injected into mammals and that it is understood by one or ordinary skill in the art that oligonucleotides are taken up by cells and tissues in animals without special delivery methods. The oligonucleotides has a sequence that forms a triple-stranded nucleic acid molecule with the target sequence located in the genome of

the intact human or animal, wherein the oligonucleotide binds to the target sequence with a Kd of less than or equal to 2 x 10-7, and mutates the target sequence. The remainder of the claim language is similar to the language in claim 7 and is supported by the '376 and '426 patents as discussed above. Therefore, claim 15 is fully supported by the disclosures of the '376 and '426 patents.

Claim 16 is supported by the '376 and '426 patents. As pointed out by the examiner on page 17 of the Office Action mailed May 10, 2005, the '376 and '426 patents disclose TFOs of 7 to 40 nucleotides (see column 4, line 4 of the '376 patent) and TFOs of 10, 20, 30 and 57 nucleotides in length. This clearly demonstrates that the '376 patent discloses TFOs of between 10 to 60 nucleotides in length as defined in claim 16.

Support for claim 17 can be found in the '376 patent at least at column 5, lines 49-50, which discloses that the oligonucleotides can be dissolved in a physiologically-acceptable carrier.

Claims 19 and 20 are supported by the '376 and '426 patents. As discussed above in regards to claim 9, the '376 patent discloses at least at column 3, lines 49-56, that TFOs can be used to stimulate recombination of a DNA fragment into a target region, but does not distinguish between whether the DNA fragment is linked or unlinked. However, at least at the paragraph spanning column 1 to column 2, the '376 patent discloses that TFOs are useful alone or linked to reactive moieties. It would be obvious to one of skill in the art that the DNA fragment described in the '376 patent at least at column 3, lines 49-56 and at column 6, lines 40-58 could be linked or unlinked to the TFO as defined by claim 20 and claim 19, respectively.

Support for claim 21 can be found in the '376 patent at least at the paragraph spanning columns 3 and 4, which describes that the double-stranded nucleic acid can be a gene, an oncogene, or a defective gene, and at column 6, lines 19-31, which describes that the double-stranded nucleic acid can be a viral genome or a portion of a viral genome.

Support for claim 22 can be found in the '376 patent at least at column 2, lines 36-38 which discloses that gene therapy is being used on an experimental basis to treat well known genetic disorders of humans such as retinoblastoma, cystic fibrosis, and sickle cell anemia. The '376 patent at least at column 6, lines 19-20 discloses that the target gene may contain a mutation that is the cause of a genetic disorder such as a defective cystic fibrosis gene and a defective hemophilia gene or hemoglobin gene as in sickle cell anemia. The examiner notes in the office action on page 18 that the '376 and '426 patents provide support for xeroderma pigmentosum gene. It was well known to one of ordinary skill in the art that many genes cause xeroderma pigmentosum (XP) and are involved in nucleotide excision repair (NER), such as xeroderma pigmentosum A (XPA), which is discussed in the '376 patent at least at column 7, lines 7-11 (see the enclosed abstract that is a review of XP and the genes that cause XP that are also involved in nucleotide excision repair; Hansson, "Inherited defects in DNA repair and susceptibility to DNA-damaging agents" *Toxicol. Lett.* 64-65 Spec No:141-148 (1992) submitted with the Amendment and Response filed July 21, 2005).

Support for claims 23 and 24 can be found in the '376 patent at least at column 4, lines 7-9, which discloses that the base composition of the oligonucleotides can be homopurine, homopyrimidine, polypurine or polypyrimidine.

In summary, the claims of the present application are **fully** supported by the '376 and '426 patents. Therefore, Chan is not prior art and claims 7-12, 15-21, and 23-25 are not anticipated by Chan.

(iv) Rejections Under 35 U.S.C. § 103

As discussed above, claim 22 is **fully** supported by the '376 and '426 patents. Therefore, claim 22 of the present application is entitled to a priority date of 1995 and therefore, Chan is not prior art. Claim 22 is not obvious in view of Chan.

(9) SUMMARY AND CONCLUSION

(1) It is well established that the specification need not contain an example if the invention is otherwise disclosed in such manner that one skilled in the art will be able to practice it without an undue amount of experimentation. *In re Borkowski*, 422 F.2d 904, 908, 164 USPQ 321, 325 (CCPA 1956). However, in this case, numerous actual examples have been provided which fully support the claimed method. The specification, in *combination* with information known in the art at the time of filing, clearly enables one skilled in the art to practice the claimed method, with a reasonable expectation of success.

Appellant has demonstrated by virtue of the examples that the *in vitro* data is predictive of the *in vivo* results. Appellant has provided results for oligonucleotides linked or unlinked to a donor nucleic acid using both cell-free and cell systems. Absent some evidence otherwise, one skilled in the art would expect the oligonucleotides linked or unlinked to a donor nucleic acid to have the same degree of efficacy in animals as in the cell systems, based on the evidence in the specification.

(2) As discussed above, the triplex forming oligonucleotides can be tethered or not to a donor nucleic acid as defined by claims 20 and 19, respectively. In view of the specification, it would be clear to one of ordinary skill in the art that the donor nucleic acid as defined in claim 7 is the same donor nucleic acid as defined in claims 19 and 20. Therefore, claims 19 and 20 are definite.

(3) Chan, published in 1999, is not prior art. Appellants have extensively demonstrated where support for each of the claims of the present application can be found in the priority documents, the '376 and '426 patents, filed long before Chan was published in 1999. The claims are fully supported by the disclosure found in the '376 and '426 patents and are therefore entitled to a priority date of 1995.

For the foregoing reasons, Appellant submits that claims 7-12 and 15-25 are patentable.

Respectfully submitted,

Patrea L. Pabst
Reg. No. 31,284

Date: September 21, 2005

PABST PATENT GROUP LLP
400 Colony Square, Suite 1200
1201 Peachtree Street
Atlanta, Georgia 30361
(404) 879-2151
(404) 879-2160 (Facsimile)

Claims Appendix: Claims On Appeal

7. A method for targeted recombination of a nucleic acid molecule comprising the steps of:

a) providing a single-stranded oligonucleotide having a sequence that forms a triple-stranded nucleic acid molecule by hybridizing with a target sequence in a double-stranded nucleic acid molecule with a Kd of less than or equal to 2×10^{-7}; and

b) providing a donor nucleic acid such that recombination of the donor nucleic acid into the target sequence is induced by triple helix formation between the single-stranded oligonucleotide and the double-stranded nucleic acid molecule.

8. The method of claim 7, wherein the single-stranded oligonucleotide is between 10 and 60 nucleotides in length.

9. The method of claim 7, wherein the single-stranded oligonucleotide is tethered to the donor nucleic acid.

10. The method of claim 7 wherein the double-stranded nucleic acid molecule encodes a protein and the targeted recombination of the donor nucleic acid with the double-stranded nucleic acid molecule alters the activity of the protein encoded by the double-stranded nucleic acid molecule.

11. The method of claim 7, wherein the double-stranded nucleic acid molecule is selected from the group consisting of a gene, an oncogene, a defective gene, a viral genome, and a portion of a viral genome.

12. The method of claim 7, wherein the donor nucleic acid is at least 30 nucleotide residues in length.

15. The method of claim 7 to produce changes in the genome of an intact human or animal wherein

the single-stranded oligonucleotide is administered into an intact human or animal having a sequence that forms a triple-stranded nucleic acid molecule with the target sequence located in the genome of the intact human or animal, wherein the oligonucleotide binds to the target sequence with a Kd of less than or equal to 2 x 10-7, and mutates the target sequence.

16. The method of claim 15 wherein the oligonucleotide is between 10 and 60 nucleotides in length.

17. The method of claim 15 wherein the oligonucleotide is dissolved in a physiologically acceptable carrier.

18. The method of claim 15 wherein the oligonucleotide is recombinagenic.

19. The method of claim 18 wherein the oligonucleotide stimulates recombination of an exogenously supplied donor nucleic acid with the target sequence of the genome.

20. The method of claim 18 wherein the oligonucleotide stimulates recombination of a donor nucleic acid that is tethered to the oligonucleotide with the target sequence of the genome.

21. The method of claim 15 wherein the target sequence is selected from the group consisting of a gene, an oncogene, a defective gene, a viral genome, and a portion of a viral genome.

22. The method of claim 21 wherein the gene is a defective -hemoglobin gene, cystic fibrosis gene, xeroderma pigmentosum gene, nucleotide excision repair pathway gene, or hemophilia gene.

23. The method of claim 15 wherein the oligonucleotide is composed of homopurine or homopyrimidine nucleotides.

24. The method of claim 15 wherein the oligonucleotide is composed of polypurine or polypyrimidine nucleotides.

25. The method of claim 9 wherein the donor nucleic acid is between 10 and 40 nucleotides.

U.S.S.N. *
Filed: *
APPEAL BRIEF

Evidence Appendix

(1) Zendegui, et al., *Nucleic Acids Research* 20:307-314 (1992).

(2) Agrawal, et al., *Proc. Natl. Acad. Sci.* USA 88, 7595 (1991).

(3) Hansson, "Inherited defects in DNA repair and susceptibility to DNA-damaging agents" *Toxicol. Lett.* 64-65 Spec No:141-148 (1992) (Abstract Only).

(4) Luo, et al., "High-frequency intrachromosomal gene conversion induced by triplex-forming oligonucleotides microinjected into mouse cells" *PNAS* 97(16):9003-9008 (2000).

Related Proceedings Appendix

None directly, although the Board's attention is drawn to the appeal in U.S.S.N. 09/783,338.

TABLE OF CONTENTS